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Isolated reduction of haematocrit does not compromise *in vitro* blood coagulation

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Low haematocrit values are generally well tolerated in terms of oxygen transport but a low haematocrit might interfere with blood coagulation. We thus sampled 60 ml of blood in 30 healthy volunteers. The blood was centrifuged for 30 min at 2000 g and separated into plasma, which contained the platelet fraction, and packed red blood cells. The blood was subsequently reconstituted by combining the entire plasma fraction with a mixture of packed red blood cells, 0.9% saline, so that the final haematocrit was either 40, 30, 20, or 10%. Blood coagulation was assessed by computerized Thrombelastograph[®] analysis. Data were compared using repeated measures analysis of variance and post-hoc paired t-tests with Bonferroni correction. Decreasing the haematocrit from 40 to 10% resulted in a shortening of reaction time (*r*) and coagulation time (*k*), and an increase in angle α , maximum amplitude (MA) and clot strength (G) (all $P < 0.02$). This pattern represents acceleration of blood coagulation with low haematocrit values. The isolated reduction in haematocrit, therefore, does not compromise *in vitro* blood coagulation.

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Avoiding allogeneic blood transfusions^{1 2} and avoiding hypovolaemia^{3 4} is important in the perioperative care of surgical patients. A treatment strategy tailored to both of these aims will result in periods of low haematocrit in patients undergoing surgical procedures associated with significant blood loss. In general, such acute haemodilution is well tolerated⁵ but there are recent publications that raise concerns that a low haematocrit might compromise blood coagulation^{6–8} and even recommend allogeneic blood transfusion to raise the haematocrit at least to 30% just to optimize blood coagulation.⁶ It is indeed known that large volume infusions of colloids progressively compromise blood coagulation.^{9–11} In contrast, moderate haemodilution with crystalloids may accelerate blood coagulation.^{9 12 13} We thus studied the effect of a progressive reduction in haematocrit *per se* on blood coagulation.

Materials and methods

With approval of the Anaesthesia and Surgery Ethics Committee of the University Hospital Zurich and written

informed consent, 30 healthy volunteers (24–51 yr of age) were enrolled into this study. Exclusion criteria were known cardiovascular, lung or liver diseases, history of bleeding diathesis, known alcohol, drug or nicotine abuse, treatment with heparin, or acetylsalicylic acid within 5 days before the study and treatment with non-steroidal anti-inflammatory drugs within 24 h of the study.

A total of 60 ml of blood was collected in six 10 ml graduated polypropylene tubes (Milian S.A., Geneva, Switzerland) containing 1 ml of buffered sodium citrate (0.129 M). Five aliquots were centrifuged for 30 min at 2000 g. The remaining aliquot served as the reference to determine the effect, if any, of blood handling, centrifugation and pipetting on blood coagulation. After centrifugation, the initial haematocrit was determined directly in the graduated tube. The blood was separated into packed red blood cells and platelet poor plasma with the entire buffy coat, i.e. plasma containing virtually all the platelets. In one of these aliquots, the blood was reconstituted without haematocrit manipulation. In the remaining four aliquots, a part of the packed red blood cells was replaced with 0.9%

saline so that the final haematocrit, after mixing with the entire plasma and platelet fraction, yielded a final haematocrit of 40, 30, 20, or 10%, respectively. Platelets, haemoglobin, and haematocrit were subsequently measured in the reconstituted blood (Coulter® Ac T8, Coulter Corporation, Miami, FL, USA).

Blood coagulation was assessed using a computerized Thrombelastograph® Coagulation Analyser (CTEG® #3000, Haemoscope, Morton Grove, IL, USA) with celite activation.¹⁴ The following variables of the TEG® trace were analysed: reaction time (*r*, normal value 12.0 (2.3) mm)—the time from the start of the recording until the amplitude reaches 2 mm; coagulation time (*k*, normal value 4.2 (1.6) mm)—the time from the end of reaction time (*r*) until the amplitude achieves 20 mm; maximum amplitude (MA, normal value 63.5 (4.5) mm) of the TEG® tracing represents the absolute strength of the clot; angle α (normal value 60.2 (6.7)°)—the angle formed by the slope of the TEG® tracing from the *r* to the *k* value. Clot lysis was represented by the percentage decrease in the area under the curve at 60 min (Lys60, normal value <15%) after MA.⁹ One millilitre of citrated blood was pipetted into a tube containing 1% celite, and 340 ml of celite-activated citrated blood was added to a TEG® cup containing 20 ml 2 M CaCl₂ for recalcification. Clot strength (G) was calculated from the MA as: $G = (5000 \times MA) / (96 - MA)$.^{15 16}

Statistical analysis

The effect of blood centrifugation, pipetting and reconstitution was assessed by comparing TEG® variables before and after processing using paired *t*-tests. The effect of progressive *in vitro* haemodilution was assessed with repeated measures ANOVA and post-hoc paired *t*-test with Bonferroni correction. Data are presented as mean (SD). *P*-values less than 0.05 were considered significant.

Results

Blood handling including centrifugation did not affect blood coagulation (Table 1).

Progressive reduction of haematocrit from 40 to 10% did not result in compromised blood coagulation as assessed by TEG® (Fig. 1). The platelet count was unaffected, reaction time (*r*) and coagulation time (*k*) decreased, and angle α and MA increased with decreasing haematocrit levels. Clot strength (G) increased with decreasing haematocrit levels. Clot lysis at 60 min decreased minimally with the reduction in haematocrit.

Discussion

The current study indicates that an isolated reduction of haematocrit does not compromise *in vitro* blood coagulation. In contrast, with decreasing haematocrit, all TEG® variables unanimously changed towards an accelerated

Table 1 Effect of blood centrifugation, pipetting and reconstitution (centrifuged) on blood coagulation. Note, there are no significant differences. Hb=haemoglobin, HCT=haematocrit *r*=reaction time, *k*=coagulation time, MA=maximum amplitude, G=clot strength and Lys60=clot lysis at 60 min

	Non-centrifuged	Centrifuged	<i>P</i>
Hb (g dL ⁻¹)	14.0 (1.0)	13.9 (1.0)	0.697
HCT (%)	40.9 (2.9)	41.0 (2.9)	0.712
Platelets (10 ⁹ litre ⁻¹)	183 (44)	179 (46)	0.472
<i>r</i> (mm)	11.6 (4.5)	11.2 (4.2)	0.473
<i>k</i> (mm)	4.6 (2.0)	4.4 (1.6)	0.492
Angle α (°)	62.0 (9.9)	61.9 (9.0)	0.914
MA (mm)	59.5 (5.0)	59.3 (4.0)	0.757
G (10 ³ dynes cm ⁻²)	8.4 (1.9)	8.3 (1.7)	0.533
Lys60 (%)	4.4 (2.0)	4.2 (1.4)	0.445

blood coagulation profile resulting in increased clot strength (G).

It was recently shown that there is virtually no thrombin generation in the absence of platelets.¹⁷ With the addition of red blood cells, however, thrombin generation gradually recovered and it was speculated that red blood cells would participate in the haemostatic process through exposure of procoagulant phospholipids at their outer cell membrane.^{17 18} In the presence of a normal number of platelets, as in the current study, *in vitro* blood coagulation appears not to be compromised significantly by a reduced number of red blood cells. Indeed, all TEG® variables indicated accelerated blood coagulation with a decreasing haematocrit resulting in an increased clot strength (G) (Fig. 1).

How relevant is this *in vitro* study for the clinical care of surgical patients? Of course, the *in vitro* situation is different from the clinical care of surgical patients, but only the *in vitro* situation allows the assessment of the isolated effect of a relevant change in haematocrit on blood coagulation. In surgical patients, low haematocrits are usually encountered after advanced surgical blood loss and asanguineous infusion therapy to maintain normovolaemia. Therefore, the patients have lost also coagulation factors and platelets. In this situation, coagulopathy will result from decreased levels of coagulation factors and platelets as well as the effects of dilution with crystalloids and colloids.⁹ Interestingly, Mezzano and colleagues found that platelet dysfunction and severity of renal dysfunction but not the haematocrit were independent predictors for haemostatic disorders in uraemic patients.¹⁹ Therefore, it may appear somewhat simplistic to advocate the use of allogeneic red blood cell transfusions for the specific purpose of optimizing blood coagulation.⁶

The use of TEG® analysis may also be criticized. We chose TEG® analysis because, in a variety of clinical situations, TEG® was the most sensitive method to predict surgical blood loss or the occurrence of bleeding complications.^{20–25} TEG® is increasingly used in studies on blood coagulation^{14 15 26} and *in vivo* and *in vitro* haemodilution results in similar TEG® changes in

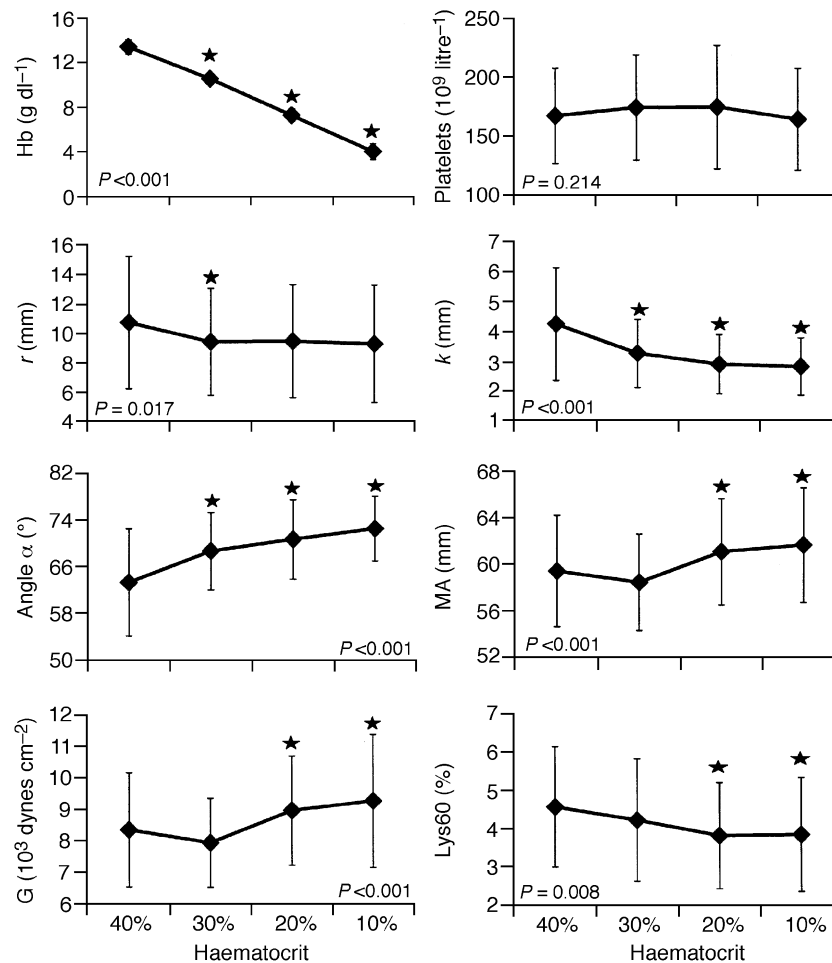


Fig 1 Effect of the isolated reduction in haematocrit from 40 to 10% on *in vitro* blood coagulation. *P* values refer to the overall ANOVA, * indicates *P*<0.017 vs haematocrit of 40%. Hb=haemoglobin, *r*=reaction time, *k*=coagulation time (*k*), MA=maximum amplitude, G=clot strength (G), and Lys60=clot lysis at 60 min

humans.²⁷ Interestingly, the results of the present study are in accordance with an *in vivo* study in rabbits demonstrating that a reduction of the haematocrit from 36 to 22% was not associated with an increased blood loss because of a subsequent standard splenic incision.⁸ The current study suggests that a reduction of haematocrit even beyond this range is not associated with significantly compromised blood coagulation. The results are also in agreement with previous findings that clot strength (G) tended to be higher in platelet rich plasma when compared with whole blood.¹⁶

The current study assessed the effect of an isolated decrease in haematocrit on *in vitro* blood coagulation with constant platelet count and unchanged coagulation factors. This is different from a previous study by Egli and colleagues,⁹ where the effect of a progressive *in vitro* haemodilution with a concomitant dilution of platelets and coagulation factors was investigated. Accordingly, different results were found. At 60% haemodilution, corresponding to a haematocrit of approximately 17%, blood coagulation was largely unchanged and MA decreased.⁹ In contrast, blood coagulation was accelerated in the current study at 20%

haematocrit with an increase in MA (Fig. 1). This may be explained by preserved platelet count and coagulation factors.

In conclusion, the isolated reduction of the haematocrit appears not to compromise *in vitro* blood coagulation and recommendations to raise the haematocrit to at least 30% with the use of allogeneic blood transfusions to optimize blood coagulation⁶ should be viewed with reservation considering the potential adverse effects of allogeneic blood transfusions.¹

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